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Sperm Motility of Brek Fish (*Systomus Orphoides*) Using Soy Extract as a Supplementation Extender

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ABSTRACT
Brek fish production (Systomus orphoides) is thought to be overexploited, recently. Accordingly, to overcome this problem, so, the broodstock male sperm must be preserved. The present study was conducted to investigate the additional soy extract as a supplement to the extender on sperm motility of brek fish 48 h after preservation. The sperm was gathered by hand stripping method and was diluted with the extender of fish ringer + 10% methanol + soy extract. The soy extract used were 20%, 15%, 10%, 5%, and 0% (control), respectively. Five replications were carried out for each group. The sample was preserved at 4-5^{o}C for 48 hours. Based on ANOVA and Tukey test, the results showed that soy extract had an effect ($p < 0.05$) on the motility of brek fish spermatozoa after preservation and the 10% concentration of soy extract was the optimum concentration (92,51 ± 4,28%).

Keywords*: Motility, preservation, soy extract, sperm, Systomus orphoides*

1. INTRODUCTION

Brek fish (*Systomus orphoides*) has a high economic value as a commercial fish [1]. Brek fish is widely traded both fresh and processed in the Purbalingga & Banyumas areas, Central Java, Indonesia. According to [2] the brek fish population has disclaimed due to over-fishing, therefore, it was to be vulnerable. To preserved so it is, necessary to preserve the offside of their habitat. However, that often occurs is the limited number of male fish in the natural habitat.

Currently, several types of local fish such as brek fish have been successfully reproduced by the Freshwater Cultivation Center and Fish Seed Center in several regions, but still require increased production because their seeds are still very limited [3]. To overcome these challenges, it is necessary to supply spermatozoa using the preservation method.

The most important factor in rising the production activities is superior broodstock with mature gonads that will produce high quanitity and quality seeds. It takes a long time and money to create excellent broodstock. As a result, the presence of a superior broodstock must be fully exploited. The sperm can be deposited throughout the reproductive season so that when needed it can be utilized immediately without using the mature male gonad anymore.

Since freshwater fish spermatozoa have a very short life span after escaping the testes, sperm storage is important. Sperm storage requires an additional supplementation extender that can act as a source of energy and protection towards low temperatures since without the supplementation extender, sperm is prone to injury and death during storage [4].

Soybean is a plant that can be used as a diluent. Soybeans contain nutrients such as protein, minerals, fats, and carbohydrates. These components are also included in semen and are needed by spermatozoa. It is hoped that the use of soy extract as an additional supplement in this extender can avoid contamination of microorganisms that can interfere with the life of spermatozoa.

Research on the the extract of soybean utilization as a natural supplementation extender for the preservation of sperm brek fish is still unknown,

therefore, the aim of this research was to assessed the effect of adding soy extract as a supplementation extender to the motility of brek fish spermatozoa during storage at 4-5 ºC for 48 h.

2. MATERIALS AND METHODS

2.1. Collection of Ejaculated Semen

Sperm ejaculation from a total of 25 broodstock male brek fish were collected by hand stripping. The ejaculate sperm was sucked neddless syringe and was stored in 1,5 ml microtube.

2.2. Fish Ringer Preparation

The preparation of fish ringer was performed by dissolving 0.0175 g/L $CaCl_2.2H_2O$, 0.0124 g/L KCl, 0.325 g/L NaCl, and 0.01 g/L $NaHCO_3$ in 100 ml distilled water.

2.3. Sperm Dillution

The dilution of sperm was done in a solution that made up from fish ringer, methanol (10%) and soy extract, with concentration of 20%, 15%, 10%, 5%, and 0%

2.4. Preservation Process

The diluted sample was stored in a 1.5 mL cryotube and preserved at 4-5 °C for 48 h.

2.5. Parameter Examined 48 Hours after Preservation

All samples were examined microscopically and macroscopically. The microscopic observations included observing sperm motility using a microscope with an eyepiece connected digitally to an image driving software on the computer, and the macroscopic observations involved observing the pH, volume, and color of the sperm.

2.6. Motility Observation (Microscopic)

The motility calculations were carried out by diluting the sperm on the fish ringers (ratio 90:10). After that, 10 µL of diluted sperm were then dropped into the Improved Neubauer counting. Motility examination was performed at 10x40 magnification. After that, the motility rate was calculated as shown below [5]:

$$M = \frac{\Sigma \text{ Motilited spermatozoa}}{\Sigma \text{ Total spermatozoa}} \text{ x } 100\% \quad (1)$$

3. RESULTS AND DISCUSSION

3.1. Macroscopically Observation

The ejaculated Fresh semen was milky white, pH 8.0, the 3.7 mLof volume total ejaculation, and 93.22 ± 2.89% fresh sperm motility (Table 1). Based on visual observations, brek fish has a pH of 8. This result was similar to research conducted by [6], using *Tor soro* (Cyprinidae) species which has a pH of 8.5, freshwater fish sperm has a pH of basic. PH is a crucial indicator in setting up the extender. The most frequently used parameter to assess sperm quality is the sperm motility. The motility sperm observation can be seen in Figure 1.

A total of 3.7 ml of fresh sperm was produced from 25 broodstock male fish with an average volume of 0.4 ml. The volume of brek fish semen was less than the research conducted by [7] using *Cyprinus carpio* (Cyprinidae) at 8.95 ± 12.95 ml. According to [8], sperm volume is influenced by several factors, including body size, put management of offering food, and sperm ejaculated frequency, which can cause differences in the volume of sperm produced.

The motility value of fresh spermatozoa was 93.22 ± 2.89%. According to [9] a statement that samples with a motility proportion of more than 70% can be used for short-term sperm storage.

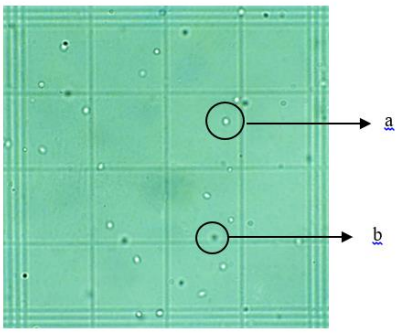


Figure 1. Motility of spermatozoa, (a) motile sperm, (b) dead sperm

3.2. Sperm Motility after Preservation

The motility of preserved sperm was lower compared with fresh sperm. The percentage of sperm motility assessed after preservation, using soy extract at a concentration of 0% (control), 5%, 10%, 15%, and 20%, were 83.83 ± 0.99%, 86.70 ± 1.17%, 92.51

± 4.28%, 91.03 ± 1.74%, and 87.63 ± 4.48%. According to the Anova test results, it shows that several concentrations of soy extract (0%, 5%, 10%, 15%, 20%) was significant effect ($P < 0.05$) for the sperm motility of brek fish 48 h after preservation. Based on the Tukey test of 10% soy extract, also displayed significant difference ($P < 0.05$). The concentration of 10% soy extract was the optimum concentration for all treatments (Figure 2).

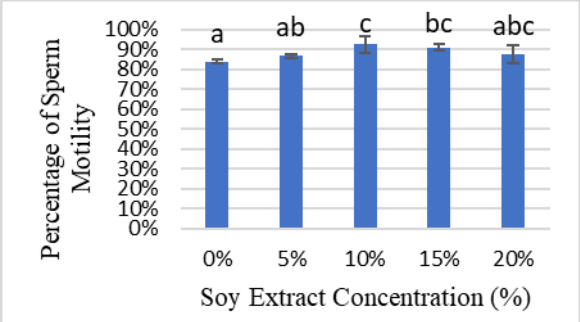
There is a difference in the motility of fresh and preserved sperm. Treatment with 10% soy extract showed the highest percentage of motility at 92.51 ± 4.28% ($p < 0.05$), similar to the study conducted by [10] the motility of goldfish species with the highest soy lecithin concentration was 10% at 45.6 ± 1.9%. [11] stated that the amount of motility reflects good sperm quality.

Soybean as an additional supplementation extender can increase the motility of the brek fish preservation. Soybean contains higher levels of lecithin than egg yolks, which tend to be contaminated by microorganisms [12]. In addition, soybeans can be used as an alternative that can replace the components of diluents that are free from animal protein. Soy extract as a supplementation extender affected the motility of brek fish spermatozoa at 4-5 ºC for 48 h.

Table 1. Motility observation fresh sperm (macroscopically and microscopically analysis) the mean ± SD from five replications

Macroscopically analysis		
Color	pH	Total Volume (mL)
Milky White	8	3.7
Microscopically analysis		
Sperm motility (%)		
93.22 ± 2.89%		

The composition contained in soybean is similar to egg yolk. The use of soy-based diluents for preserving spermatozoa can produce quality that may be better than egg yolks which are prone to contamination by microorganisms. The lecithin content in soybeans can be the right choice as an additional supplement for extender in semen dilution.



Significant different among treatments were shown by different notation ($P>0.05$) Tukey test

Figure 2. Histogram of sperm motility after preservation

4. CONCLUSION

It is known that soy extract has an effect on the motility of brek fish spermatozoa and 10% of soy extract is the optimum concentration as an extender supplementation on the motility of brek fish spermatozoa at 92.51 ± 4.28%.

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